

# A New Species of *Rhacophorus* (Amphibia: Anura: Rhacophoridae) from Vietnam

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**Abstract** We describe a new species of *Rhacophorus* based on two adult specimens collected from Hoa Binh Province, northern Vietnam. *Rhacophorus hoabinhensis* **sp. nov.** is distinguishable from its congeners on the basis of a combination of the following morphological characters: size small (SVL 31.1–32.5 mm in males); head slightly longer than wide; vomerine teeth absent; snout short (SNL/SVL 0.16); dorsal skin smooth; forearm and tarsus with dermal fringes; dermal appendage at vent present; webbing formula on fingers I12/3–12/3III1–2III1–1IV and on toes I3/4–1III1/2–1III1/2–1IV1–1/2V; dorsal surface grey yellow with brown spots; lower jaw region dark grey, throat, chest and belly cream; anterior and posterior thighs, as well as ventral surface of tibia orange. The interspecific uncorrected genetic distances (16S rRNA gene) between the new species from Hoa Binh and other analyzed congeners varied from 9.8% to 17.4%. In the phylogenetic analyses, the new species revealed to be a representative of *Rhacophorus* and was nested within the *R. hoanglienensis-orlovi* species group.

**Keywords** *Rhacophorus hoabinhensis* **sp. nov.**, karst forest, molecular phylogeny, taxonomy, Hoa Binh Province

## 1. Introduction

The genus *Rhacophorus* is one of the most diverse genera of amphibians, with 90 recognized species and a wide distribution from India throughout China and the Indochina region, eastwards to Japan and southwards to the Philippines (Frost, 2017). In Vietnam, a total of 22 species of *Rhacophorus* has been recorded and ten of them have been recently described since 2000 (Ohler

*et al.*, 2000; Ziegler and Köhler, 2001; Ohler and Delorme, 2006; Orlov, 2008; Orlov *et al.*, 2001, 2012; Ostroshabov *et al.*, 2013; Rowley *et al.*, 2010, 2012). Nguyen *et al.* (2014) indicated that the members of *Rhacophorus* from Vietnam represent two major clades, however, the phylogenetic relationships of some species within each clade remained unresolved, including the small-sized species of the *Rhacophorus hoanglienensis-orlovi* species group (Ostroshabov *et al.*, 2013).

During our recent field work in northwestern Vietnam, specimens of a small treefrog species were collected in the karst forest of Hang Kia-Pa Co Nature Reserve in Hoa Binh Province. These treefrogs could be placed in the genus *Rhacophorus* both based on molecular and

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morphological data (Brown and Alcala, 1994; Duellman and Trueb, 1986; Liem, 1970). Morphologically, the specimens from Hoa Binh resemble *R. orlovi* Ziegler and Köhler, 2001 in size and color pattern. However, closer morphological examination showed that they bear a suite of distinct features, also compared to the other known members of *Rhacophorus*. Further, the phylogenetic analyses revealed the treefrogs from Hoa Binh to be separate from their congeners and to be clustered within the *R. hoanglienensis-orlovi* species group with a strong support value. Based on these results, we describe the *Rhacophorus* from Hoa Binh Province herein as a new species.

## 2. Materials and Methods

**2.1 Sampling** Field surveys were conducted from 8 to 20 April 2015 by C. T. Pham, H. N. Ngo, T. V. Nguyen (hereafter C. T. Pham *et al.*) in Hang Kia-Pa Co Nature Reserve, Mai Chau District, Hoa Binh Province, northwestern Vietnam. Specimens were collected between 19:00 and 23:30. After taking photographs, specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons, 2002), fixed in 80% ethanol for four hours, and then later transferred to 70% ethanol for permanent storage. Tissue samples were preserved separately in 70% ethanol. Preserved specimens were deposited in the collections of the Institute of Ecology and Biological Resources (IEBR) and the Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam.

**2.2 Molecular data and phylogenetic analyses** We used the protocols of Kuraishi *et al.* (2013), modified by Nguyen *et al.* (2015), for DNA extraction, amplification, and sequencing. Fragments of three mitochondrial DNA genes (12S rRNA, tRNA<sup>val</sup>, and 16S rRNA) were amplified using the primers following Kuraishi *et al.* (2013).

In addition to newly generated sequences, we compiled available data from 18 other species of *Rhacophorus* from GenBank (Table 1). *Buergeria buergeri* (Temminck and Schlegel) was used as outgroup based on Nguyen *et al.* (2014).

Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using MAFFT version 7 (Katoh and Standley, 2013) with default settings. We then checked the initial alignments by eye and adjusted slightly. Phylogenetic trees were constructed by using maximum likelihood (ML) and Bayesian inference

(BI). Prior to ML and Bayesian analyses, we chose the optimum substitution models for entire sequences using by Kakusan 4 (Tanabe 2011) based on the Akaike information criterion (AIC). The best model selected for ML was the general time reversible model (GTR: Tavaré 1986) with a gamma shape parameter (G: 0.306 in ML and 0.359 in BI). The BI summarized two independent runs of four Markov Chains for 10 000 000 generations. A tree was sampled every 100 generations and a consensus topology was calculated for 70 000 trees after discarding the first 30 001 trees (burn-in = 3 000 000). We checked parameter estimates and convergence using Tracer version 1.5 (Rambaut and Drummond, 2009). The strength of nodal support in the ML tree was analyzed using non-parametric bootstrapping (MLBS) with 1000 replicates. We regarded tree nodes in the ML tree with bootstrap values of 75% or greater as sufficiently resolved (Huelsenbeck and Hillis, 1993), and nodes with a BPP of 95% or greater as significant in the BI analysis (Leaché and Reeder, 2002). Pairwise comparisons of uncorrected sequence divergences (*p*-distance) were calculated for 16S rRNA fragments only between species of the genus *Rhacophorus*.

**2.3 Morphological characters** Measurements were taken with a digital caliper to the nearest 0.1 mm. Terminology for morphological characters followed Nguyen *et al.* (2012, 2016). Abbreviations are as follows: SVL: Snout-vent length, HL: Head length (from back of mandible to tip of snout), HW: Maximum head width (across angle of jaws), SNL: Snout length (from anterior corner of eye to tip of snout), NS: Distance from nostril to tip of snout, EN: Distance from anterior corner of eye to nostril, IN: Internarial distance, IOD: Interorbital distance (minimal distance between orbits), ED: Eye diameter, UEW: Maximum width of upper eyelid, DAE: Distance between anterior corner of eyes, DPE: Distance between posterior corner of eyes, MAE: Distance between angle of jaw and anterior corner of the eye, MPE: Distance between angle of jaw and posterior corner of the eye, MN: Distance from angle of jaw to nostril, TYD: Maximal tympanum diameter, TYE: Distance from anterior margin of tympanum to posterior corner of eye, FLL: Forearm length (from axilla to elbow), HAL: from elbow to tip of third finger, fd1-4: Width of discs of fingers I-IV, fw1-4: Maximal width of fingers I-IV, TFL: Third finger length, OPT: Outer palmar tubercle length, FeL: Thigh length (from vent to knee), TbL: Tibia length (from knee to tarsus), TbW: Maximal tibia width, FoL: Foot length (from tibiotarsal joint to tip of fourth toe), FTL: Fourth toe length, IMT: Inner metatarsal tubercle length, OMT:

**Table 1** Samples of Vietnamese *Rhacophorus* and other species used for DNA analysis in this study together with information on voucher, collection locality and GenBank accession numbers. Voucher abbreviations: AMNH = American Museum of Natural History; AMS = Australian Museum; CIB = Chengdu Institute of Biology; IABHU = Institute for Amphibian Biology, Hiroshima University; IEBR = Institute of Ecology and Biological Resources; KIZ = Kunming Institute of Zoology; KUHE = Graduate School of Human and Environmental Studies, Kyoto University; NCSM = North Carolina Museum of Natural Sciences; SCUM = Sichuan University Museum; UNS = University of Science, Ho Chi Minh City; VNMN = Vietnam National Museum of Nature.

Sample No.	Species	Voucher	Locality	Accession No.	Reference
1	<i>R. annamensis</i>	VNMN 4092	Vietnam, Gia Lai, Kon Ka Kinh	LC010568	Nguyen <i>et al.</i> , 2014
2	<i>R. annamensis</i>	AMNH A161414	Vietnam, Quang Binh, Cha Lo	DQ283047	Frost <i>et al.</i> , 2006
3	<i>R. annamensis</i>	VNMN 4090	Vietnam, Dak Nong, Nam Nung	LC010566	Nguyen <i>et al.</i> , 2014
4	<i>R. bipunctatus</i>	KUHE 53375	Malaysia, Pahang, Genting	LC010569	Nguyen <i>et al.</i> , 2014
5	<i>R. bipunctatus</i>	VNMN 4117	Vietnam, Gia Lai, Kon Ka Kinh	LC010604	Nguyen <i>et al.</i> , 2014
6	<i>R. calcaneus</i>	VNMN 4093	Vietnam, Dak Lac, Chu Yang Sin	LC010573	Nguyen <i>et al.</i> , 2014
7	<i>R. dennysi</i>	SCUM 060401L	China, Guangdong, Shaoguan	EU215545	Li <i>et al.</i> , 2008
8	<i>R. dennysi</i>	VNMN 4098	Vietnam, Vinh Phuc, Tam Dao	LC010576	Nguyen <i>et al.</i> , 2014
9	<i>R. dorsovireidis</i>	VNMN 4099	Vietnam, Son La, Ta Xua	LC010577	Nguyen <i>et al.</i> , 2014
10	<i>R. duboisi</i>	VNMN 4102	Vietnam, Lao Cai, Sa Pa	LC010580	Nguyen <i>et al.</i> , 2014
11	<i>R. duboisi</i>	VNMN 4103	Vietnam, Lao Cai, Sa Pa	LC010581	Nguyen <i>et al.</i> , 2014
12	<i>R. dugritei</i>	SCUM 051001L	China, Sichuan, Baoxing	EU215541	Li <i>et al.</i> , 2008
13	<i>R. dugritei</i>	CIB 20050194	China, Sichuan	LC010583	Nguyen <i>et al.</i> , 2014
14	<i>R. exechopygus</i>	VNMN 4107	Vietnam, Gia Lai, Kon Ka Kinh	LC010585	Nguyen <i>et al.</i> , 2014
15	<i>R. exechopygus</i>	VNMN 4108	Vietnam, Gia Lai, Kon Ka Kinh	LC010586	Nguyen <i>et al.</i> , 2014
16	<i>R. feae</i>	VNMN 3462	Vietnam, Lao Cai, Sa Pa	LC010588	Nguyen <i>et al.</i> , 2014
17	<i>R. feae</i>	SCUM 050642W	China, Yuan Nam	EU215544	Li <i>et al.</i> , 2008
18	<i>R. helenae</i>	AMS R 173230	Vietnam, Binh Thuan	JQ288087	Rowley <i>et al.</i> , 2012
19	<i>R. helenae</i>	UNS 00450	Vietnam, Dong Nai	JQ288088	Rowley <i>et al.</i> , 2012
20	<i>R. hungfuensis</i>	SCUM 060425 L	China, Sichuan, Wenchuan	EU215538	Li <i>et al.</i> , 2008
21	<i>R. hungfuensis</i>	SCUM 060424 L	China, Sichuan, Wenchuan	JN688879	Li <i>et al.</i> , 2012
22	<i>R. kio</i>	VNMN 4110	Vietnam, Gia Lai, Kon Ka Kinh	LC010589	Nguyen <i>et al.</i> , 2014
23	<i>R. kio</i>	VNMN 4111	Vietnam, Ha Giang, Bac Quang	LC010590	Nguyen <i>et al.</i> , 2014
24	<i>R. maximus</i>	VNMN 1534	Vietnam, Bac Giang, Yen Tu	LC010592	Nguyen <i>et al.</i> , 2014
25	<i>R. maximus</i>	VNMN 4113	Vietnam, Nghe An, Pu Huong	LC010593	Nguyen <i>et al.</i> , 2014
26	<i>R. minimus</i>	KIZ 061214YP	China, Yuan Nam	EU215539	Li <i>et al.</i> , 2008
27	<i>R. minimus</i>	CIB GX2009.05.11	China, Yuan Nam	LC010594	Nguyen <i>et al.</i> , 2014
28	<i>R. nigropunctatus</i>	SCUM 070657L	China, Guizhou	EU215533	Li <i>et al.</i> , 2008
29	<i>R. orlovi</i>	VNMN 3067	Vietnam, Ha Tinh, Huong Son	LC010598	Nguyen <i>et al.</i> , 2014
30	<i>R. orlovi</i>	VNMN 4116	Vietnam, Kon Tum, Ngoc Linh	LC010599	Nguyen <i>et al.</i> , 2014
31	<i>R. orlovi</i>	VNMN 4115	Vietnam, Nghe An, Pu Huong	LC010600	Nguyen <i>et al.</i> , 2014
32	<i>R. rhodopus</i>	SCUM 060692L	China, Jinghong	EU215531	Li <i>et al.</i> , 2008
33	<i>R. robertingeri</i>	VNMN 4123	Vietnam, Gia Lai, Kon Ka Kinh	LC010613	Nguyen <i>et al.</i> , 2014
34	<i>R. robertingeri</i>	VNMN 3446	Vietnam, Kon Tum, Kon Plong	LC010615	Nguyen <i>et al.</i> , 2014

(Continued Table 1)

Sample No.	Species	Voucher	Locality	Accession No.	Reference
35	<i>R. hoabinhensis</i> <b>sp.nov.</b>	IEBR A.2016.18	Vietnam, Hoa Binh	LC331096	This study
36	<i>R. hoabinhensis</i> <b>sp.nov.</b>	VNMN A.2016.16	Vietnam, Hoa Binh	LC331097	This study
37	<i>R. spelaeus</i>	IEBR A.2011.1	Lao, Khammouan	LC331095	This study
38	<i>R. vampyrus</i>	NCSM 77318	Vietnam, Lam Dong, Bidoup-Nui Ba	HQ656815	Rowley <i>et al.</i> , 2010
39	<i>R. vampyrus</i>	VNMN 4125	Vietnam, Khanh Hoa, Hon Ba	LC010616	Nguyen <i>et al.</i> , 2014
40	<i>Buergeria buergeri</i>	IABHU 41011	Japan, Hiroshima	AB127977	Sano <i>et al.</i> , 2004

Outer metatarsal tubercle length, td1-4: Maximal width of discs of toes I-IV, tw1-4: Width of toes I-IV. Webbing formula terminology followed Glaw and Vences (1997). Sex was determined by the presence of nuptial pads and gonadal inspection.

We compared the new species with 45 congeners from Vietnam and neighboring countries in mainland Indochina, including Laos, Cambodia, Thailand and southern China based on specimen examination (see Appendix 1) and data obtained from the literature (Bain and Nguyen, 2004; Bourret, 1937, 1942; Chan and Grismer, 2010; Fei *et al.*, 2009, 2010; Inger *et al.*, 1999; Liu and Hu, 1960; Liu *et al.*, 2012; Matsui and Panha, 2006; Mo *et al.*, 2008, 2016; Nguyen *et al.*, 2008; Ohler *et al.*, 2001; Ohler and Delorme, 2006; Ostroshabov *et al.*, 2013; Orlov, 2008; Orlov *et al.*, 2001, 2009, 2012; Taylor, 1962; Rowley *et al.*, 2010, 2012; Ziegler and Köhler, 2001).

### 3. Results

**Phylogenetic analyses** Aligned, combined sequences of 12S rRNA, tRNA<sup>val</sup>, and 16S rRNA yielded a total of 2607 characters. Of 2607 nucleotide sites, 1066 were variable and 852 were parsimony informative within the in-group. The ML and Bayesian analyses produced topologies with  $-\ln L = 14\,813.335$  and  $16\,180.015$ , respectively.

Phylogenetic analyses employing ML and BI methods yielded slightly different topologies only among referenced species, and only the ML tree is presented in Figure 1.

Monophyly of the genus *Rhacophorus* with respect to the outgroup species was supported (100 and 100% support in ML bootstrap value and Bayesian posterior probability, respectively), and the genus was split into two major clades. The Clade A comprised four subclades A1, A2, A3 and A4. The subclade A1 contained *R. orlovi*,

*R. robertingeri*, *R. spelaeus*, and the *Rhacophorus* species from Hoa Binh with strong support values (MLBS = 99%, BPP = 1.00) (Figure 1).

The interspecific uncorrected genetic *p*-distances (16S rRNA gene) examined between the unnamed *Rhacophorus* species from Hoa Binh and all analysed congeners varied from 9.8% (compared with *R. orlovi*) to 17.4% (compared with *R. exechopygus* and *R. vampyrus*) (Table 2). Thus, we describe the species of *Rhacophorus* from Hoa Binh Province, Vietnam as a new species in the following:

***Rhacophorus hoabinhensis* sp. nov.**

**Holotype** IEBR A.2016.18 (Field No. HB 2015.7), adult male, collected by C. T. Pham *et al.* on 9 April 2015 in karst forest near Hang Kia Village (20°44.020' N, 104°53.370' E, at an elevation of 1350 m), Hang Kia Commune, Mai Chau District, Hoa Binh Province, Vietnam (Figures 2–4).

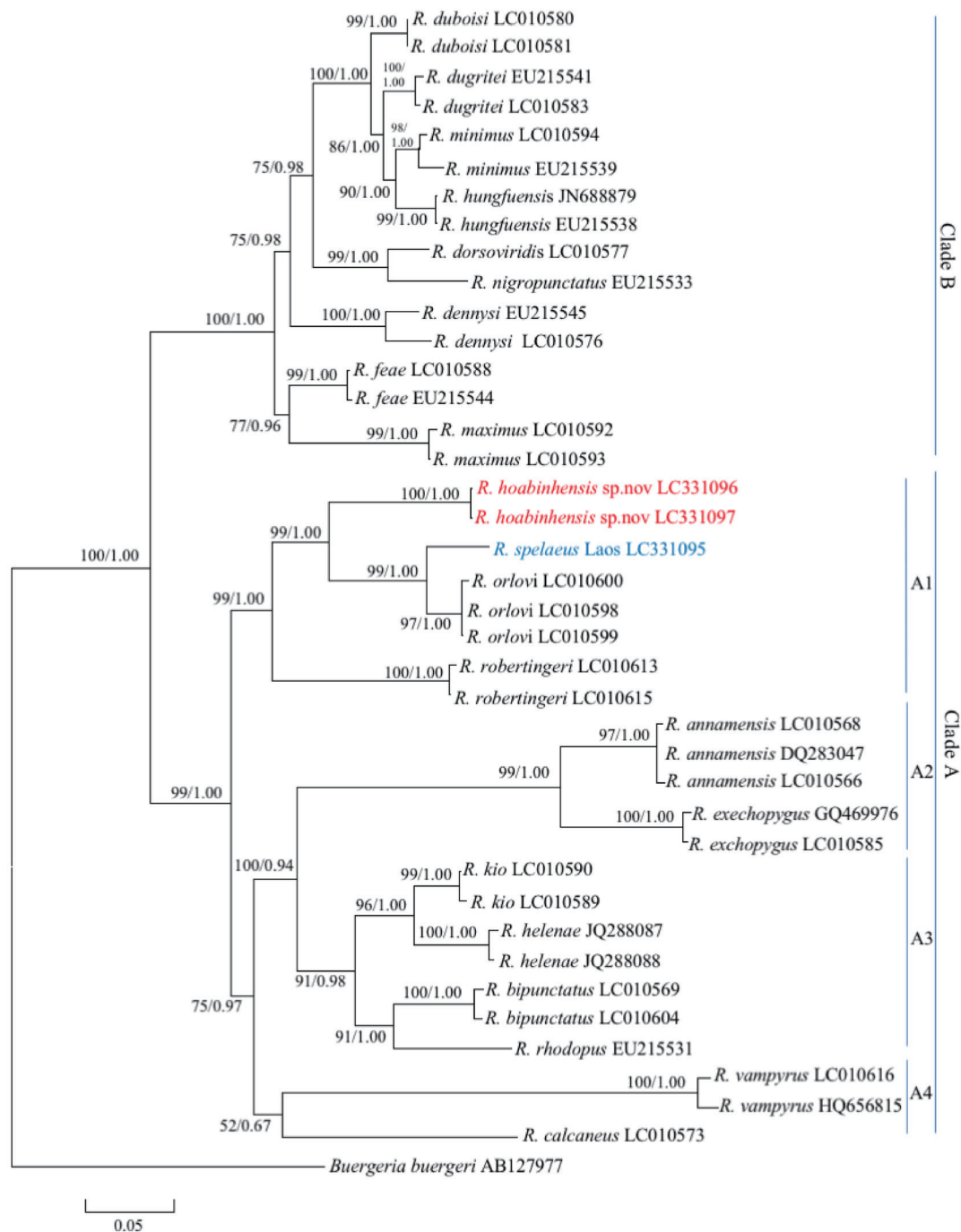
**Paratype** VNMN A.2016.16 (Field No. HB 2015.24), adult male, collected by C.T. Pham *et al.* on 11 April 2015 in karst forest near Hang Kia Village (20°44.400' N, 104°53.893' E, at an elevation of 1230 m), Hang Kia Commune, Mai Chau District, Hoa Binh Province, Vietnam.

**Diagnosis** The new species is attributed to the genus *Rhacophorus* on the basis of the following morphological characters (after Brown and Alcalá, 1994; Duellman and Trueb, 1986; Liem, 1970): presence of intercalary cartilage between the terminal and penultimate phalanges of digits, Y-shaped distal end of terminal phalanx, tips of digits expanded into large disks bearing circum marginal grooves, webbed fingers, a supracloacal dermal ridge, and pupil horizontal. The new species is distinguished from its congeners and other small rhacophorid species by a combination of the following characters: 1) Size small (SVL 31.1–32.5 mm in males); 2) head longer than wide;

**Table 2** Mean uncorrected (“*p*”) distance (%) among 549 bp fragments of 16S rRNA from species of the genus *Rhacophorus*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. <i>Rhacophorus hoabinhensis</i> sp. nov.	–																				
2. <i>R. annamensis</i>	11.0	–																			
3. <i>R. bipunctatus</i>	15.3	10.6	–																		
4. <i>R. calcanus</i>	12.8	12.8	14.2	–																	
5. <i>R. demysi</i>	12.5	12.9	13.5	15.4	–																
6. <i>R. dorsoviridis</i>	11.0	12.3	15.1	14.7	8.4	–															
7. <i>R. duboisi</i>	12.5	14.2	15.9	15.8	8.1	8.0	–														
8. <i>R. dugritiei</i>	10.6	13.0	15.1	14.6	7.7	6.2	3.2	–													
9. <i>R. exechopygus</i>	17.4	8.6	12.2	13.3	15.0	14.5	15.0	14.6	–												
10. <i>R. feae</i>	9.9	9.9	13.0	11.5	7.3	4.9	6.2	5.9	12.5	–											
11. <i>R. helenae</i>	12.9	12.0	9.9	15.7	13.0	13.8	13.9	13.1	14.9	12.5	–										
12. <i>R. hungfuensis</i>	10.6	13.0	15.1	15.3	8.4	6.6	3.2	2.6	14.6	6.2	13.0	–									
13. <i>R. kio</i>	14.9	12.1	9.5	16.0	13.8	14.6	14.0	12.8	14.5	14.6	5.5	13.2	–								
14. <i>R. maximus</i>	11.7	12.9	14.3	15.4	9.9	9.1	7.0	8.0	15.6	6.6	13.9	8.4	15.2	–							
15. <i>R. minimus</i>	11.0	13.8	15.1	15.4	8.1	5.9	3.9	1.3	15.0	6.3	13.5	2.9	14.4	7.7	–						
16. <i>R. nigropunctatus</i>	11.6	12.2	14.2	14.5	9.1	4.2	9.4	7.6	14.8	6.6	11.9	8.6	14.3	10.2	8.0	–					
17. <i>R. orlovi</i>	9.8	12.1	15.6	13.2	15.6	14.5	14.6	13.4	16.3	12.5	14.6	13.4	13.8	14.8	14.5	14.7	–				
18. <i>R. rhodopus</i>	16.0	12.7	8.7	15.8	13.9	15.9	17.1	17.0	14.1	12.7	12.3	17.0	12.7	17.0	17.4	15.7	14.4	–			
19. <i>R. robertingeri</i>	12.7	12.9	15.2	14.1	14.0	14.9	13.8	13.3	17.2	11.4	15.6	13.8	14.9	13.4	14.2	16.0	12.3	16.5	–		
20. <i>R. speltaeus</i>	10.3	14.5	16.4	14.0	16.2	15.0	15.1	14.6	17.2	12.9	15.2	14.7	16.0	15.8	15.8	16.4	3.5	15.3	12.0	–	
21. <i>R. vampyrus</i>	16.9	15.0	15.5	18.2	14.6	17.1	17.5	17.1	18.7	15.3	16.4	16.7	16.5	16.2	18.3	17.9	17.8	20.4	17.9	18.4	–





**Figure 1** Maximum likelihood (ML) tree based on partial sequence of mitochondrial genes (12S rRNA, tRNA<sup>val</sup>, and 16S rRNA) for samples of *Rhacophorus* and reference species. Numbers above and below branches represent bootstrap support for ML and Bayesian posterior probabilities (MLBS/BPP).

3) vomerine teeth absent; 4) snout short (SNL/SVL 0.16); 5) dorsal skin smooth; 6) dermal fringe on outer edges of forearm and tarsus present; 7) dermal appendage at vent present; 8) webbing formula on fingers: I12/3-12/3II1-2III1-1IV and on toes I3/4-1III1/2-1III1/2-1IV1-1/2V; 9) dorsal surface of head and body yellowish grey with dark

brown blotches; 10) lower jaw region dark grey, throat, chest and belly cream; and 11) anterior and posterior thighs, as well as ventral surface of tibia orange.

**Description of holotype** Adult male, body robust (SVL 32.5 mm), dorsoventrally compressed. Head slightly



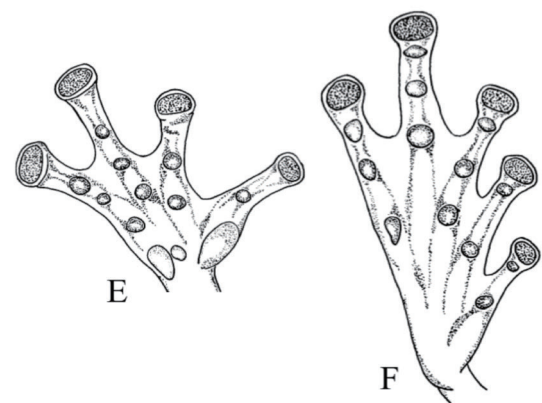
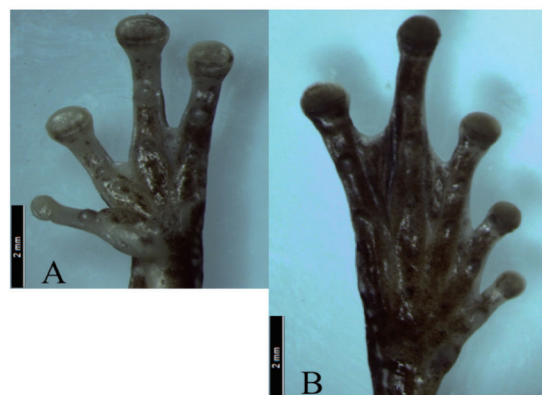
**Figure 2** Dorsal and ventral views of the adult male holotype (IEBR A.2016.18) of *Rhacophorus hoabinhensis* **sp. nov.** from Hoa Binh Province, northern Vietnam.

longer than wide (HL 13.1 mm, HW 12.1 mm), convex above; snout pointed anteriorly from dorsal view, slightly protruding beyond lower jaw in lateral view, its length longer than horizontal diameter of eye; canthus rostralis round, loreal region oblique, concave; interorbital distance wider than internarial distance and upper eyelid; distance between anterior corners of eyes about 64% of distance between posterior corners of eyes; nostril round, without lateral flap of skin, closer to tip of snout than to eye; pupil oval, horizontal; tympanum distinct, round, smaller than eye diameter, greater than distance between tympanum and eye; pineal ocellus absent; spinules on upper eyelid absent; vomerine teeth absent; choanae small, oval; tongue heart-shaped, deeply notched posteriorly; vocal sacs absent; supratympanic fold distinct, extending from behind eye to beyond level of axilla.

**Forelimbs** Arm short, about half of lower arm and hand length (FLL 6.8 mm, HAL 16.5 mm), dermal fringe along outer edge of forearm present but not well developed; relative lengths of fingers  $I < II < V < III$ ; tips of fingers with enlarged discs with distinct circum marginal grooves; disc of finger III approximately two times of width of finger

III (fd3/fw3 1.99) but smaller than tympanum diameter (fd3/TYD 1.02); webbing formula I12/3-12/3III1-2III1-1IV; subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 2; nuptial pads prominent, oval, smooth.

**Hindlimbs** Heels overlapping when held at right angles to the body; tibia length about five times longer than tibia width (TbL 17.1 mm, TbW 3.1 mm), longer than

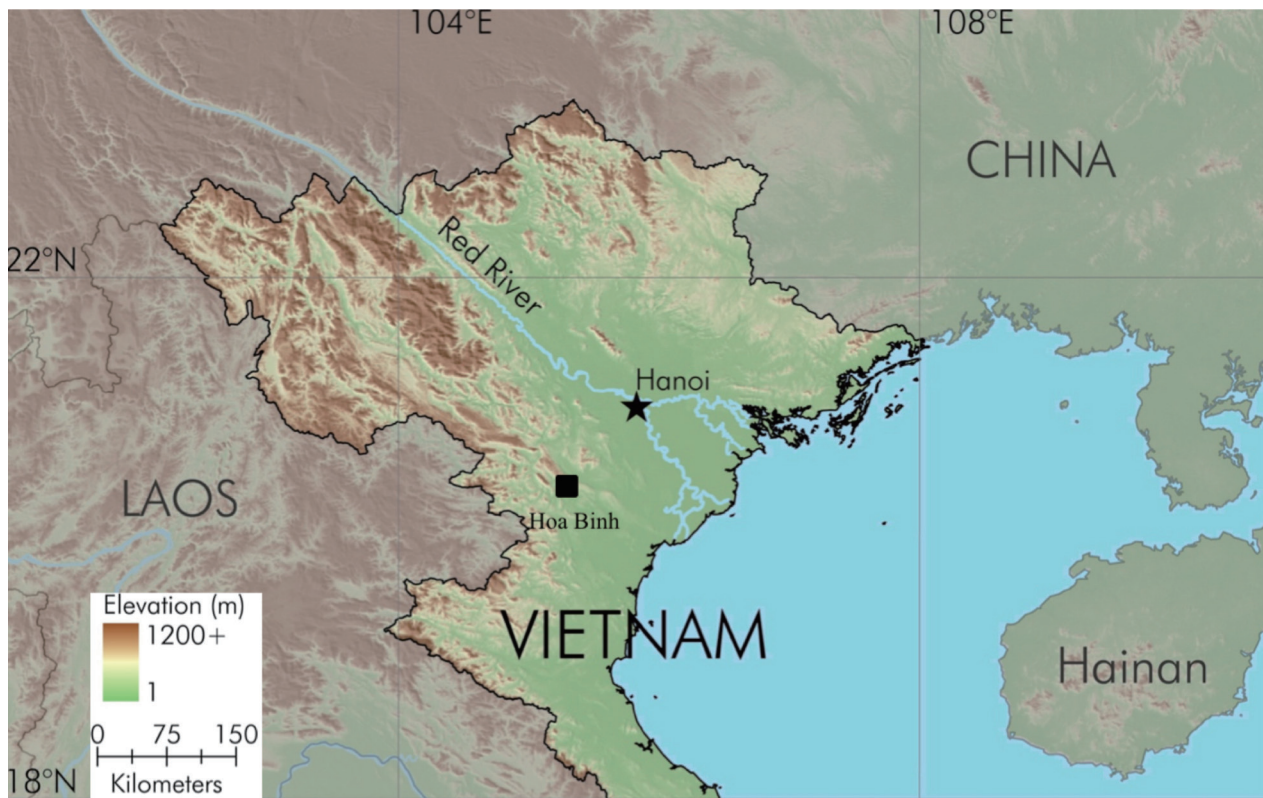


**Figure 3** Ventral views of left hand (A) and right foot (B) of the male holotype (IEBR A.2016.18) of *Rhacophorus hoabinhensis* **sp. nov.** in preservative. Scale bar is 2 mm; (C) left hand and (D) right foot of the holotype of *R. orlovi* (from Ziegler and Köhler, 2001); (E) right hand and (F) right foot of the holotype of *R. hoanglienensis* (from Orlov *et al.*, 2001).





**Figure 4** *Rhacophorus hoabinhensis* **sp. nov.** in its biotope in Hang Kia–Pa Co Nature Reserve, Hoa Binh Province, Vietnam.



**Figure 5** Type locality (black square) of *Rhacophorus hoabinhensis* **sp. nov.** in Hoa Binh Province, Vietnam.



thigh length (FeL 16.1 mm) but shorter than foot length (FoL 23.1 mm); relative lengths of toes  $I < II < III < V < IV$ ; tips of toes with enlarged discs with distinct circum marginal grooves, discs slightly smaller than those of fingers; webbing formula  $I3/4-1III1/2-1III1/2-1IV1-1/2V$ ; subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 3, 2; inner metatarsal tubercle small (IMT 1.0 mm); dermal ridge along outer edge of tibia and tarsus present; dermal projection at tibiotarsal articulation present.

**Skin texture** Dorsal surface of head and body smooth; flanks, dorsal surface and lateral sides of limbs with small granules; dorsolateral folds absent; throat and chest smooth, belly granular; ventral surface of fore and hind limbs smooth; dermal appendage at vent present.

**Coloration in life** Iris greyish brown, pupil horizontal black; background of dorsal surface yellowish grey with dark brown blotches; head with a triangular brown blotch between eyes; tympanum brown, supratympanic fold dark brown with white granules; dorsum and upper part of flanks yellowish grey with white granules; dorsal surface of fore and hind limbs yellowish grey with dark brown bars; ventral surface of arms and thighs yellow, anterior and posterior parts of thighs, and ventral surface of tibia reddish orange; lower jaw region dark grey, throat, chest and belly cream; posterior part of belly translucent; dermal fringes on outer edges of fore and hind limbs white, nuptial pads yellow; webbing yellow grey.

**Coloration in preservative** Dorsal surface of head, dorsum, and upper part of flanks grey with dark blotches; upper lips and tympanum dark brown; dorsal surface of fore limbs, thigh, tibia and foot grey with dark bands, posterior part of thigh below the vent light brown with small white dots; lower jaw region gray; chin brown; throat, chest and belly cream; ventral part of forelimbs white; ventral surface of thighs white with small brown spots; webbing brown.

**Variation** Measurements and morphological characters of the type series are given in Table 3. The paratype is slightly smaller than the holotype.

**Comparisons** *Rhacophorus hoabinhensis* sp. nov. differs from *R. burmanus* (Andersson), *R. dennysi* Blanford, *R. duboisi* Ohler, Marquis, Swan and Grosjean, *R. feae* (Boulenger), *R. helenae* Rowley, Tran, Hoang and Le, *R. kio* Ohler and Delorme, *R. maximus* Günther, *R. nigropalmatus* Boulenger, *R. norhayatii* Chan and Grismer, and *R. prominans* Smithby having a significantly smaller size in males (SVL 31.1–32.5 mm vs.  $\geq 60$  mm in other species), and different dorsal

coloration (yellowish grey with brown blotches vs. green background with or without white spots); from *R. calcaneus* Smith, *R. chenfui* Lui, *R. dorsoviridis* Bourret, *R. dugritei* David, *R. hungfuensis* Liu and Hu, *R. hongchibaensis* Li, Liu, Chen, Wu, Murphy, Zhao, Wang and Zhang, *R. hui* Liu, *R. leucofasciatus* Liu and Hu, *R. minimus* Rao, Wilkinson and Liu, *R. nigropunctatus* Liu, Hu and Yang, *R. omeimontis* Stejneger, *R. puerensis* He, *R. yaoshanensis* Liu and Hu, *R. yinggelingensis* Chou, Lau and Chan, and *R. wui* Li, Liu, Chen, Wu, Murphy, Zhao, Wang and Zhang by having dorsal color yellowish grey with brown blotches (vs. green dorsum in others species); from *R. annamensis* Smith by having a smaller size in males (SVL 31.1–32.5 mm vs.  $\geq 60$  mm in *R. annamensis*), vomerine teeth absent (vs. present in *R. annamensis*), different webbing on fingers and toes (webbing formula  $I12/3-12/3III1-2III1-1IV$  and  $I3/4-1III1/2-1III1/2-1IV1-1/2V$  vs. almost fully webbed in *R. annamensis*); from *R. bipunctatus* Ahl by having a smaller size in males (SVL 31.1–32.5 mm vs. 65 mm in *R. bipunctatus*), vomerine teeth absent (vs. present in *R. bipunctatus*), different dorsal color pattern (yellowish grey vs. reddish brown in *R. bipunctatus*), and different webbing on fingers and toes (webbing formula  $I12/3-12/3III1-2III1-1IV$  and  $I3/4-1III1/2-1III1/2-1IV1-1/2V$  vs. almost fully webbed in *R. bipunctatus*); from *R. cyanopunctatus* by the absence of vomerine teeth (vs. present in *R. cyanopunctatus*) and different webbing on fingers and toes (webbing formula  $I12/3-12/3III1-2III1-1IV$  and  $I3/4-1III1/2-1III1/2-1IV1-1/2V$  vs. almost fully webbed in *R. cyanopunctatus*); from *R. exechopygus* Inger, Orlov and Darevsky by having a smaller size in males (SVL 31.1–32.5 mm vs. 45–50 mm in *R. exechopygus*), vomerine teeth absent (vs. present in *R. exechopygus*), and different webbing on fingers and toes (webbing formula  $I12/3-12/3III1-2III1-1IV$  and  $I3/4-1III1/2-1III1/2-1IV1-1/2V$  vs. almost fully webbed in *R. exechopygus*); from *R. hoanglienensis* Orlov, Lathrop, Murphy and Ho by having a smaller size in males (SVL 31.1–32.5 mm vs. 41–56 mm in *R. hoanglienensis*), vomerine teeth absent (vs. present in *R. hoanglienensis*), different dorsal color pattern (yellowish grey with brown spots vs. chocolate brown or light beige with small black dots in *R. hoanglienensis*), and different webbing on fingers and toes (webbing formula  $I12/3-12/3III1-2III1-1IV$  and  $I3/4-1III1/2-1III1/2-1IV1-1/2V$  vs.  $I1-11/4III1/2-11/2III1-1IV$  and  $I1-1III0-1III0-1IV1-0V$ , respectively in *R. hoanglienensis*), different iris color (greyish brown vs. brownish-yellow or beige in *R. hoanglienensis*); from *R. laoshan* Mo, Jiang, Xie and Ohler by having a smaller

**Table 3** Sex, measurements (in mm) and ratios of *Rhacophorus hoabinhensis* sp. nov.

Variables	IEBR A.2016.18	VNMN A.2016.16	Variables	IEBR A.2016.18	VNMN A.2016.16
	Holotype	Paratype		Holotype	Paratype
Sex	♂	♂	HAL	16.5	16.3
SVL	32.5	31.1	IPT	1.7	1.7
HL	13.1	12.5	FeL	16.1	15.2
HW	12.1	12.0	TbL	17.1	16.2
MN	11.4	10.8	TbW	3.1	3.3
MFE	9.1	8.3	FoL	23.1	21.6
MBE	4.3	3.9	HL/SVL	0.40	0.40
SNL	5.1	5.0	HW/SVL	0.37	0.39
ED	4.7	4.6	HL/HW	1.08	1.03
UEW	3.1	3.0	ED/SNL	0.92	0.92
IN	3.6	3.6	TYD/ED	0.47	0.49
IOD	3.9	3.8	SNL/SVL	0.16	0.16
DAE	7.5	7.2	NS/EN	0.75	0.73
DPE	11.8	11.4	fd3/TYD	1.02	1.05
NS	2.4	2.5	FLL/SVL	0.5	0.5
EN	3.2	3.1	HAL/SVL	5.4	4.9
TYD	2.2	2.0	TbL/SVL	0.5	0.5
TYE	0.6	0.5	FoL/SVL	0.7	0.7
FLL	6.8	5.9	TbL/TbW	5.4	4.9

size in males (SVL 31.1–32.5 mm vs. 33–37 mm in *R. laoshan*), different dorsal coloration (grey yellow with brown spots vs. chocolate brown in *R. laoshan*), vomerine teeth absent (vs. present in *R. laoshan*); from *R. larissae* Ostroshabov, Orlov and Nguyen by having a smaller size in males (SVL 31.1–32.5 mm vs. 49.9 mm in *R. larissae*), vomerine teeth absent (vs. present in *R. larissae*), and different dorsal coloration (yellowish grey with brown blotches vs. chocolate brown in *R. larissae*), anterior and posterior parts of thighs, and ventral surface of tibia reddish orange (vs. brown in *R. larissae*); from *R. marmoridorsum* Orlov by having a smaller size in males (SVL 31.1–32.5 mm vs. 50 mm in *R. marmoridorsum*), vomerine teeth absent (vs. present in *R. marmoridorsum*), different dorsal color pattern (yellowish grey with brown blotches vs. flesh-colored, cream-colored with rough edges along the center of the back in *R. marmoridorsum*), and different webbing on toes (webbing formula I3/4-1III1/2-1III1/2-1IV1-1/2V vs. I0-1/2II0-1III0-1IV1-0V in *R. marmoridorsum*); from *R. orlovi* Ziegler and Köhler by

having a smaller size in males (SVL 31.1–32.5 mm vs. 37–45 mm in *R. orlovi*), vomerine teeth absent (vs. present in *R. orlovi*), different dorsal coloration (yellowish grey vs. reddish brown in *R. orlovi*), and different webbing on fingers and toes (webbing formula I12/3-12/3II1-2III1-1IV and I3/4-1III1/2-1III1/2-1IV1-1/2V vs. I11/4-11/4II3/4-11/2III11/4-3/4IV and I0-1II0-1III0-1V1-0V); from *R. jarujini* Matsui and Panha by having a smaller size in males (SVL 31.1–32.5 mm vs. 38 mm in *R. jarujini*), different dorsal color pattern (yellowish grey vs. light brown in *R. jarujini*), and different webbing on fingers and toes (webbing formula I12/3-12/3II1-2III1-1IV and I3/4-1III1/2-1III1/2-1IV1-1/2V vs. almost fully webbed in *R. jarujini*); from *R. pinglongensis* Mo, Chen, Liao and Zhou by the absence of vomerine teeth (vs. present in *R. pinglongensis*), different dorsal color pattern (yellowish grey with brown blotches vs. green in *R. pinglongensis*), different color pattern on anterior and posterior surfaces of thighs (reddish orange vs. black blotches with white spots in *R. pinglongensis*); from *R.*

*rhodopus* Liu and Hu by having vomerine teeth absent (vs. present in *R. rhodopus*), different dorsal color pattern (yellowish grey vs. reddish brown in *R. rhodopus*), and different webbing on fingers and toes (webbing formula I12/3-12/3III1-2III1-1IV and I3/4-1III1/2-1III1/2-1IV1-1/2V vs. almost fully webbed in *R. rhodopus*); from *R. robertingeri* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Nguyen and Geissler by having a smaller size in males (SVL 31.1–32.5 mm vs. 37–44 mm in *R. robertingeri*), vomerine teeth absent (vs. present in *R. robertingeri*), different dorsal color pattern (yellowish grey with brown blotches vs. red-brown tones with orange spots in *R. robertingeri*), and different webbing on toes (webbing formula I3/4-1III1/2-1III1/2-1IV1-1/2V vs. I1/2-1/2II0-0III0-1/2IV1/2-0V in *R. robertingeri*); from *R. robinsoni* by the absence of vomerine teeth (vs. present in *R. robinsoni*), and different webbing on toes (webbing formula I3/4-1III1/2-1III1/2-1IV1-1/2V vs. fully webbed in *R. robinsoni*); from *R. spelaeus* Orlov, Gnophanxay, Phimminith and Phomphoumy by having a smaller size in males (SVL 31.1–32.5 mm vs. 38.9–43.1 mm in *R. spelaeus*), vomerine teeth absent (vs. present in *R. spelaeus*), different dorsal color pattern (yellowish grey with brown blotches vs. grey brown in *R. spelaeus*), different webbing on toes (I3/4-1III1/2-1III1/2-1IV1-1/2V vs. I0-1II0-1/2III1-0V1/2-1/2V in *R. spelaeus*); from *R. translineatus* Wu by having a smaller size in males (SVL 31.1–32.5 mm vs. 52–59 mm in *R. translineatus*), and different webbing on toes (I3/4-1III1/2-1III1/2-1IV1-1/2V vs. almost fully webbed in *R. translineatus*) from *R. tuberculatus* (Anderson) by having a smaller size in males (SVL 31.1–32.5 mm vs. 45 mm in *R. tuberculatus*), and smooth dorsal skin (vs. with a big tubercle in *R. tuberculatus*); from *R. vampyrus* Rowley, Le, Tran, Stuart and Hoang by having a smaller size in males (SVL 31.1–32.5 mm vs. 42–45 mm in *R. vampyrus*), head slightly longer than wide (vs. head shorter than wide in *R. vampyrus*), different dorsal color pattern (yellowish grey with brown blotches vs. pale copper-brown in *R. vampyrus*), anterior and posterior parts of thighs, and ventral surface of tibia reddish orange (vs. anterior and posterior part of thighs, and ventral surface of tibia black in *R. vampyrus*); from *R. verrucopus* Huang by having a smaller size in males (SVL 31.1–32.5 mm vs. 37 mm in *R. verrucopus*) and different webbing on toes (I3/4-1III1/2-1III1/2-1IV1-1/2V vs. I1/4-1/2II0-1III1/4-1IV1-0V in *R. verrucopus*); from *R. viridimaculatus* Ostroshabov, Orlov and Nguyen by having a smaller size in males (SVL 31.1–32.5 mm vs. 41–57 mm in *R. viridimaculatus*), vomerine teeth absent (vs. present in *R. viridimaculatus*),

different dorsal color pattern (yellow grey vs. greyish brown in *R. viridimaculatus*), and different webbing on fingers (webbing formula I12/3-12/3III1-2III1-1IV vs. I1-1III0-1III1-1/2IV in *R. viridimaculatus*); and from *R. zhokaiyae* Pan, Zhang, Wan, Wu, Kang, Qian, Li, Zhang, Chen, Rao, Jiang, Zhang by the absence of vomerine teeth (vs. present in *R. zhokaiyae*), different dorsal color pattern (yellowish grey with brown blotches vs. green in *R. zhokaiyae*), and different iris color (greyish brown vs. golden-yellow in *R. zhokaiyae*).

**Etymology** The specific epithet “hoabinhensis” refers to the type locality of the new species, Hoa Binh Province. For the common names we suggest Hoa Binh Treefrog (English), andẾch cây hòa bình (Vietnamese).

**Distribution** *R. hoabinhensis* is currently known only from the type locality in Hang Kia–Pa Co Nature Reserve, Hoa Binh Province, Vietnam.

**Natural history** *Rhacophorus hoabinhensis* appears to be closely associated with karstic environment. Specimens were found at night between 19:00 and 23:30h, near a small pond. The surrounding habitat was secondary karst forest, consisting of medium and small hardwoods mixed with shrubs and vines. Specimens were found on leaves and branches of trees, about 1.2–1.5 m above the ground. The advertisement call of the species was not heard. The air temperatures at the times of collection ranged from 14.8 to 18.9°C and relative humidity from 84% to 93%. Other rhacophorid species recorded in Hang Kia–Pa Co Nature Reserve were *Raorchestes parvulus* Boulenger, *R. feae* Boulenger, *R. kio* Ohler and Delorme, *R. orlovi* Ziegler and Köhler, *Theloderma albopunctatum* Liu and Hu, *T. gordonii* Taylor, and *T. lateriticum* Bain, Nguyen and Doan. Females and the tadpole of *Rhacophorus hoabinhensis* have not been recorded so far.

#### 4. Discussion

Based on morphological characters, Ostroshabov *et al.* (2013) assigned eight species from China, Laos, and Vietnam with a reddish brown or greyish brown dorsum and a SVL less than 60 mm to the *Rhacophorus* “hoanglienensis-orlovi” complex, namely *R. hoanglienensis*, *R. laoshan*, *R. larissae*, *R. orlovi*, *R. rhodopus*, *R. robertingeri*, *R. vampyrus*, and *R. viridimaculatus*. In our phylogenetic analyses, two moderately supported clades were recognized. This also agreed with Li *et al.* (2008, 2009, 2012), however, the *R. calcaneus* mentioned by Li *et al.* 2012 was reassigned to *R. robertingeri* and *R. chuyangsinensis* was synonymized



with *R. calcaneus* after Orlov *et al.*, 2012. *R. hoabinhensis* was clustered in the same clade with *R. orlovi*, *R. spelaeus* and *R. robertingeri* (99% and 100% support), but it was separated from *R. calcaneus* and *R. rhodopus* (genetic divergence ranged from 12.8% to 16%).

In terms of habitat preference, *R. hoabinhensis* and *R. spelaeus* are found in karst forests in northern Vietnam and central Laos, whereas *R. robertingeri* is adapted to montane evergreen forest in central Vietnam. *R. orlovi* inhabits both habitat types in central Vietnam and central Laos (Nguyen *et al.*, 2009; Ostroshabov *et al.*, 2013). Our new finding brings the species number of *Rhacophorus* in Vietnam to 23. Further studies are required to explore the actual diversity and phylogenetic relations of treefrogs in the country.

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## Appendix 1

Specimens examined for morphological comparisons.

*Rhacophorus annamensis*: Vietnam: Quang Binh Province: Le Thuy District IEBR 3972–3974 (Field No. QB 2015.36, QB 2015.102, QB 2015.151); Vietnam: Gia Lai Province: Kon Ka Kinh National Park: VNMN 4090, IEBR 3986–3988 (KKK 2012.94–96).

*R. calcaneus*: Vietnam: Lam Dong Province: Bi Doup National Park: IEBR 3989, IEBR 3990 (BĐ 2013.6, BĐ 2013.7); Vietnam: Khanh Hoa Province: Hon Ba Nature Reserve: VNMN 4093.

*R. dennysi*: Vietnam: Cao Bang Province: Ha Lang District: IEBR 3870, IEBR 3871.

*R. dorsovireidis*: Vietnam: Son La Province: Ta Xua District: VNMN 4099.

*R. duboisi*: Vietnam: Ha Giang Province: IEBR A. 2013.100, ZFMK 9548; Vietnam: Lao Cai Province: Hoang Lien National Park: VNMN 4102, VNMN 4103.

*R. exechopygus*: Vietnam: Quang Binh Province: Le Thuy District: IEBR 3975–3977 (QB.2015.123, QB.2015.135, QB.2015.150); Vietnam: Gia Lai Province: Kon Ka Kinh National Park: VNMN 4107, VNMN 4108.

*R. feae*: Vietnam: Lao Cai Province: Hoang Lien National Park: VNMN 4109; Vietnam: Hoa Binh Province: Hang Kia–Pa Co Nature Reserve: HB 2014.28; Viet Nam: Kon Tum Province: Kon Plong District: IEBR 3991, IEBR 3992 (KT.2014.9, KT.2014.10).

*R. kio*: Vietnam: Cao Bang Province: Ha Lang District: IEBR 3872–3877; Vietnam: Gia Lai Province: Kon Ka Kinh National Park: VNMN 4110; Vietnam: Hoa Binh Province: Ngoc Son–Ngo Luong: IEBR 3978, IEBR 3979 (HB.2015.97, HB.2015.98).

*R. maximus*: Vietnam: Bac Giang Province: Tay Yen Tu NR: IEBR 3653, IEBR 3680, VNMN 1535, VNMN 1538; Vietnam: Cao Bang Province: Ha Lang District: IEBR 3878; Vietnam: Nghe An Province: Pu Huong Nature Reserve: VNMN 4113.

*R. orlovi*: Vietnam: Ha Tinh Province: Huong Son District: VNMN 3067; Vietnam: Nghe An Province: Pu Huong Nature Reserve: VNMN 4115; Vietnam: Hoa Binh Province: IEBR 3980–3983 (HB 2015.19, HB 2015.23, HB 2015.51, HB 2015.52).

*R. puerensis*: Vietnam: Lao Cai Province: Bat Xat District: IEBR 3993, IEBR 3994 (HVC 2013.23, HVC 2013.33).

*R. rhodopus*: Vietnam: Bac Giang Province: Tay Yen Tu Nature Reserve: IEBR A.2013.81, VNMN A. 2013.15, ZFMK 92855; Vietnam: Thanh Hoa: Xuan Lien Nature Reserve: IEBR A. 2015.58–61; Vietnam: Lam Dong Province: Bi Doup National Park: IEBR 3984, IEBR 3985 (BĐ 2013.27, BĐ 2013.28).

*R. robertingeri*: Vietnam: GiaLai Province: Kon Ka Kinh National Park: VNMN 4124; Vietnam: Kon Tum Province: Kon Plong District: VNMN 3446.

*R. vampyrus*: Vietnam: Lam Dong Province: Bi Doup National Park: IEBR 3995 (BĐ 2013.29); Vietnam: Khanh Hoa Province: Hon Ba Nature Reserve: VNMN 4125.